REMARKS

Claims 1-21, 23-37, 40-44, and 46-61 are pending in the application. Claims 28-37 and 40-44 were withdrawn from consideration, pursuant to a Restriction Requirement. Claims 11, 23, and 48 were objected to, and claims 1-21, 23-27, and 46-61 were rejected. The objections and rejections are addressed below.

Before addressing the objections and rejections, Applicants would again like to emphasize that the claimed methods involve detection of the presence of both proANP and proBNP, or fragments thereof, in a single reading, in a single assay. Central to the invention is that a single assay provides a single reading indicating the presence of proANP and proBNP or fragments thereof, without distinguishing the individual presence of proANP and proBNP (or fragments thereof) in the sample. The invention is based on the discovery that detection of the presence of proANP and proBNP in a single reading is sufficient to determine activation or inactivation of the ANP and BNP hormonal systems. Such an approach provides substantial benefits with respect to ease of use and efficiency and, as discussed further below, is not taught or suggested in the prior art. Rather, any teachings of detecting both ANP and BNP-related proteins in the art involves obtaining at least two readings, in more than one assay. Such teachings provide no suggestion or motivation to carry out the present methods.

Objections

Claim 11 was objected to for failing to limit the subject matter of claim 3, from which it depends. In response, Applicants have amended claim 3 to specify a "fusion polypeptide agent or a fusion peptide agent," rather than only a "fusion polypeptide agent." Claim 11 specifies that the method of claim 3 comprises use of a "fusion peptide agent," thus limiting the scope of claim

3 to one of the two options noted above. Claim 11 thus limits the scope of claim 3, and this objection should be withdrawn.

Claim 23 was objected to for including the phrase "...comprising sequence...". This objection has been addressed by amending this phrase to specify "...comprising a sequence..." consistent with the Examiner's suggestion.

Claim 48 was objected to for reciting the same limitations as claim 5, from which it depends. In response, claim 48 has been canceled, without prejudice.

Rejection under 35 U.S.C. § 101

Claim 26 was rejected under 35 U.S.C. § 101 on the basis that the claim reads on host cells that could be in a human. This claim has been amended to specify that the host cells are isolated, consistent with the Examiner's suggestion. Applicants thus request that this rejection be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-17, 46-48, 52-55, and 59-61 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite on several grounds, which are addressed as follows.

Claim 1 was rejected for omitting an essential step. In particular, the Examiner states that claim 1 does not recite a step indicating how those skilled in the art can conclude whether there is an increase or decrease in the peptides of interest. In response, Applicants have amended claim 1 to specify comparison to a reference level. Support for this amendment can be found at, for example, paragraph [0127] of the publication of the application. As stated in paragraph [0127], a reference level can be normal reference levels in the general population, and detection

of a qualitatively or quantitatively higher peptide level than the reference level can indicate activation of the ANP and BNP systems. New claim 64 has been added to specify this type of reference level. As stated in paragraph [0128], a reference level can also be a previous reading from a given subject, such as in an instance where serial assays are carried out to detect for example a response to medical treatment. New claim 62 has been added to specify this type of reference level. Further with respect to the use of reference levels, and as described in paragraph [0127], Applicants note that an assay can be calibrated so that a particular reading in the assay is known to represent a normal peptide level, and/or so that a normal level produces a negligible or insignificant amount. In these cases, the reference level is not measured directly at the same time as the test level. New claims 65 and 66 have been added to specify this type of reference level. No new matter has been added.

The Examiner also states that the term "proportionally cumulative amount" in claims 1 and 61 is indefinite. In the interest of expediting prosecution, this term has been deleted from claim 1. Claim 61 has been canceled.

Claims 3 and 47 were rejected as being incomplete for omitting essential steps, on the basis that it would not be possible to distinguish between the presence of ANP and BNP related peptides that are present in the sample as a result of activation or inactivation of the hormonal systems, in comparison to the agent. The rejection further states that these claims fail to recite method steps directed to detection of atrial and brain natriuretic peptide prohormones that are present in the sample and distinguished from the peptides of the added agent. In response, claims 3 and 47 have been amended to specify that the fusion polypeptide agent and the fusion peptide agent are used as competitive inhibitors or calibration agents, as described in paragraphs [0134] and [0315] of the application. Applicants thus respectfully request that this rejection be

withdrawn.

Claim 17 was rejected for indefiniteness on the basis that it does not specify whether an increase or decrease in ANP and BNP levels is indicative of heart failure or monitoring of treatment. In response, claim 17 has been amended to specify that detection of activation of the ANP and BNP hormonal systems is diagnostic of heart failure, and that detection of inactivation of the ANP and BNP hormonal systems monitors successful treatment of a cardiac condition.

Claim 55 was rejected for lacking antecedent basis for reference to the homologous sequence of (c) in claim 48. Claims 48 and 55 have been canceled, so this rejection is moot.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-17, 46-48, 52-55, and 59-61 were rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. Claims 1-18, 21, 23-27, and 46-61 were also rejected under 35 U.S.C. § 112, first paragraph for lack of adequate written description. Applicants respectfully request reconsideration and withdrawal of these rejections.

A central concern in these rejections is the possibility that use of a myriad of sequences is being covered by the claims. As described below, the present amendments substantially reduce the number of sequences that can be used in the claimed methods.

Applicants first note that the Examiner has set forth section A in the enablement rejection, which focuses on ANP and BNP related sequences, and section B, which focuses on GC-A and related sequences. As is provided in the amendment set forth above, references to GC-A and related sequences have been deleted from the claims and, thus, the Examiner's comments with respect to these sequences are not addressed.

Also, the Examiner commented on Applicants' use of terms relating to percentage

identity in the claims. Such terms have been deleted from the claims and, thus, the Examiner's comments with respect to these terms have not been addressed.

What remains in the rejection are the Examiner's comments with respect to Applicants' references to fragments and species homologues or allelic variants, which are addressed as follows.

In regard to fragments, Applicants note that the claims have been amended to specify that the first binding substance binds to full length sequences of (i) proANP, ANP, or NT-proANP, and (ii) proBNP, BNP, or NT-proBNP. Thus, in addition to the possibility of binding to a fragment of one of each of these sets of proteins (the ANP and the BNP-related sequences), the binding substance also must bind to a full length ANP and BNP-related sequence. Similarly, the method claims have been amended to specify that the fusion polypeptide agent or fusion peptide agent binds to a binding substance that binds to a full length sequences noted above.

Applicants further note that, as is understood in the art, a binding substance, such as an antibody, does not recognize an entire protein. Rather, it binds to and thus recognizes only a small part of the protein (i.e., an epitope). Because of this, it is reasonable to include fragments, in the claims, as that is what is actually being recognized in the context of the full length sequence. This is not overly broad, as the only fragments that are covered are those that are recognizable in the context of the full length sequences as well, in view of the above-described amendment.

Further in the rejection, the Examiner refers to teachings in the art of fragments having sequences that are 100% identical to at least 6 amino acid fragments of SEQ ID NOs:3 or 6, to support the position that Applicants have not provided guidance as to how those skilled in the art could determine false positives generated by detection of such fragments, and detection of ANP

and BNP fragments.

In response, Applicants first note that the fragments of the present claims must be at least 12 amino acids in length, rather than 6, as considered by the Examiner in this analysis. In particular, and referring to claim 3 as an example, the specified fusion polypeptide agent or fusion peptide agent comprises a fragment of both an ANP-related sequence (part (a) of the claim) and a fragment of a BNP-related sequence (part (b) of the claim), wherein each fragment is at least 6 amino acids in length, resulting in a fusion polypeptide agent or a fusion peptide agent of at least 12 amino acids in length. Therefore, the information concerning sequences of only 6 amino acids in length is not relevant to the present claims. In addition, as noted above, the binding substances of the invention are required to bind full-length sequences, in addition to possibly binding fragments.

In regard to species homologues or allelic variants, Applicants have amended the claims to specify that such homologues and variants are naturally occurring. Support for this amendment can be found, for example, at paragraph [0109] of the publication of the present application. Applicants also have added claim 68, which specifies that the test subject is a human. This amendment is supported at, for example, paragraph [0135] of the publication of the present application. Applicants respectfully submit that undue experimentation would not be required to use such homologues and variants in the present methods. In particular, once it was decided to carry out the present method with a particular subject or group of subjects, the relevant sequences of the subject or group of subjects could be determined (if not already known) and used in the present methods. In view of the above, Applicants respectfully request reconsideration and withdrawal of these rejections.

Applicants also respectfully submit that the present invention is not based on the

discovery of a new gene or protein. Rather, the invention relates to new methods and reagents that can be used to carry out the methods, which reagents can be made based on known or readily identifiable sequences. In view of this and the above-described amendments, Applicants respectfully submit that those of skill in the art would consider that the present inventors were in possession of the invention to the extent of the full scope of the claims.

Rejections under 35 U.S.C. § 103(a)

Claims 1, 16, 17, and 61 were rejected under 35 U.S.C. § 103(a) for obviousness over Clerico et al., J. Endoc. Invest. 21:170-179, 1998, in view of Clerico et al., Clin. Chemistry 46:1529-1534, 2000. Applicants request that this rejection be reconsidered and withdrawn.

Before addressing the cited references, Applicants note that, as discussed above, claim 1 requires a single reading, in a single assay of the presence of proANP and proBNP, or fragments thereof. Central to the invention is that a single assay provides a single reading indicating the presence of proANP and proBNP, without distinguishing the individual presence of proANP and proBNP in the sample. The invention is based on the discovery that detection of the presence of proANP and proBNP in a single reading is sufficient to determine activation or inactivation of the ANP and BNP hormonal systems. Such an approach, which provides substantial benefits with respect to ease of use and efficiency, is not taught or suggested in the cited references. Rather, the references require at least two readings to obtain information with respect to both ANP and BNP-related sequences.

Clerico (1998) was cited for teaching the measurement of plasma ANP and BNP individually in patients with heart failure. Clerico (2000) was cited for teaching that cardiac natriuretic hormones are a family of related peptides, including ANP, BNP, and N-terminal

portions of proANP and proBNP, which may be present in greater amounts in plasma than ANP and BNP. The Examiner concludes that it would have been obvious to use the methods of Clerico (1998) to detect the different natriuretic protein forms taught by Clerico (2000), particularly in view of the teaching of Clerico (2000) of the higher concentrations of these forms in plasma. The Examiner further concludes that "one of ordinary skill in the art, aware that it is routine to detect multiple compounds in a single sample at the same time in the performance of clinical assays. . . would be motivated to assay both ANP and BNP in the same assay to increase the efficiency and reduce to the costs of said assays."

However, nowhere does either Clerico (1998) or Clerico (2000) teach or suggest the measurement of the presence of proANP and proBNP in a single reading, in a single assay. The M.P.E.P § 2141.02 VI states "a prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984)" (emphasis original).

When considered as a whole, Clerico (1998) provides a rationale for measuring ANP and BNP *separately* as "the data reported in Figure 3 suggest that the BNP assay is more useful than the ANP assay for discriminating between normal subjects and patients with cardiomyopathy, even including those with only mild symptoms" (page 176, column 1). Furthermore, Clerico (2000) states

> [A]lthough ANP and BNP bind to the same specific receptors, they have different types of metabolism and spectra of biological activity, and their production and secretion may be regulated differently in humans. It has been suggested that there may be different pools of intracellular natriuretic peptides that can respond separately to the same hemodynamic events (e.g., overload for ANP) or to the same pathology-related factors (e.g., cardiac hypertrophy for BNP). (Page 1530, column 1).

Based on these statements, Clerico (1998) and Clerico (2000) teach the desirability of distinguishing between ANP and BNP levels and, therefore, teach away from the claimed methods, which require a single reading, in a single assay, showing the presence of proANP and proBNP, without distinguishing between the two polypeptides.

The rejection of claims 1, 16, and 17 for obviousness based on Clerico (1998) and Clerico (2000) should be withdrawn, because it would not have been obvious to those skilled in the art to measure proANP and proBNP using a method that does not distinguish between the two polypeptides, such as that now claimed.

Claims 2-4, 7-15, 46, 47, 52-54, 59, and 60 were rejected for obviousness over Clerico (1998), in view of Clerico (2000), and further in view of Buechler et al., U.S. Patent No. 7,341,838.

The Clerico references were cited for the reasons discussed above. Buechler ('838) was cited for describing amino acid sequences bearing similarity to SEQ ID NOs:3 and 6, which are stated by Buechler ('838) to correspond to proANP and proBNP. The Examiner states that those of skill in the art would have recognized that antibodies that recognize the sequences of Buechler ('838) would also recognize the sequences of the present claims, and that Buechler ('838) teaches measuring the amounts of ANP and BNP-related fragments by using antibodies, including bivalent antibodies. In view of these teachings, the Examiner concludes that it would have been obvious to modify the methods of Clerico (1998 and 2000) by substituting the sequences taught by Buechler ('838) and utilizing bispecific antibodies, as taught by Buechler ('838). Applicants respectfully disagree and request that this rejection be reconsidered and withdrawn.

As discussed above, a central feature of the present invention is the detection of the

presence of both proANP and proBNP-related sequences in a single reading, in a single assay. Also as discussed above, it would not have been obvious in view of either Clerico reference to perform a single assay to obtain a single reading that determines the presence of proANP and proBNP, without distinguishing between the two polypeptides. Buechler ('838) does not add what is missing from the Clerico references in supporting this rejection, as Buechler ('838) does not teach or suggest testing for the presence of proANP and proBNP-related sequences in a single reading, in a single assay. In view of the above, Applicants request that this rejection be reconsidered and withdrawn.

Claims 5, 6, 48, and 55 were rejected for obviousness over Clerico (1998), in view of Clerico (2000) and Buechler (U.S. Patent No. 7,341,838), and further in view of Bentivegna et al., WO 01/79231. Claims 5, 6, 48, and 55 have been canceled herein, without prejudice, rendering this rejection moot.

Claims 18-21, 27, 49, 51, 56, and 58 were rejected for obviousness over Burnett et al., U.S. Patent No. 6,818,619, in view of Buechler et al., U.S. Patent No. 7,341,838; and claims 23-26, 50, and 57 were rejected for obviousness over Burnett et al., U.S. Patent No. 6,818,619; Buechler et al., U.S. Patent No. 7,341,838; Lewicki et al., U.S. Patent No. 5,212,286; and Simari, WO 00/71576.

Even though the '619 patent discloses natriuretic peptides and chimeric peptides, the present invention would not have been obvious over the '619 patent, in view of the '836 patent, or the '619 and '836 patents in view of the '286 patent and the '576 patent application publication. None of these documents teach or suggest that a fusion polypeptide should be used in an assay, such as an immunoassay, for determining the presence of proANP and proBNP (and related proteins and peptides) in a sample.

The Examiner states that those of skill in the art would have been motivated to make the fusion polypeptides of the present invention, and furthermore to modify the teachings of the '619 and '838 patents, which teach a chimeric protein comprising ANP and BNP and expression cassettes comprising polynucleotides encoding the chimeric or fusion proteins, and substitute the polynucleotide taught by the '286 patent. However, there is no reason why those of skill in the art would have been motivated to make an ANP-BNP chimeric polypeptide. The purpose of the '619 patent was solely to provide a composition having natriuretic, rennin-suppressing, diuretic, and/or vasodilator activity, and which is useful to prevent or treat cardiovascular disorders such as congestive heart failure, and further to provide a method for inducing natriuresis, diuresis, or vasodilation in a mammal, which method is useful for treating heart failure. In order to make an efficient compound, they used a *Dendroaspis* natriuretic peptide (DNP) isolated from the venom of the green mamba snake.

The Examiner states, on page 46, last paragraph to page 47, first paragraph, that the '619 patent does not teach a fusion polypeptide agent including any of the components as recited in claims 18-20, and especially not a fusion polypeptide agent comprising proBNP1-108 and proANP1-126. The second paragraph refers to the '838 patent. However, the '838 patent discloses polypeptide sequences that are known in the art. Further, it is not the sequences but the fusion polypeptide to be used as a diagnostic means utilizing the sequences, which comprises the present invention. This argument also pertains to the other fusion polypeptide combinations of the present claims.

Column 2, line 66 to column 3, line 11 of the '619 patent discloses a chimeric peptide combining the core ring structure of BNP with the C-terminus of DNP (BD-NP), and the core ring structure of CNP with the C-terminus of DNP (CD-NP). BD-NP has a combined effect *in*

vivo, which includes potent vasodilation with a focus on pulmonary vasodilation, natriuresis, and suppression of rennin. The administration of BD-NP significantly increases the glomerular filtration rate in mammals.

Accordingly, the '619 patent teaches away from making chimeric polypeptides comprising ANP and BNP (or any other combinations of the present invention), as the focus of the '619 patent is to provide compounds having more potent natriuretic, diuretic, and/or vasodilator activity than available compounds. This would not have encouraged those of skill in the art to make chimeric polypeptides comprising ANP and BNP, but rather would have discouraged them to do so, by encouraging them to find new effective compounds. The '619 patent also does not disclose a chimeric polypeptide between ANP-CNP or between BNP-CNP, because the purpose was to develop a compound that is as efficient as possible by using DNP. If anything, this would have motivated those of skill in the art to search for compounds with efficient natriuretic, diuretic, or vasodilator activity to be fused to ANP or BNP.

Accordingly, as the '619 patent would have discouraged those of skill in the art to make chimeric polypeptides including proANP and proBNP, ANP and BNP, or NT-proANP and NT-proBNP, there certainly would not have been any reason why the '619 patent would have motivated those of skill in the art to make fusion polypeptides to be used in an assay, such as an immunoassay, for determining the presence of proANP, proBNP, ANP, BNP, NT-proANP or NT-proBNP in a sample, according to the present invention. The question is why someone skilled in the art would have been motivated to construct a fusion protein including, for example, proANP and proBNP, after having analyzed the '619 patent, in view of the '838 patent, and in view of the '286 patent and furthermore in view of the '576 patent application publication. The '619 patent provides a method for inducing natriuresis, dieresis, or vasodilation in mammals, but

not teaching or suggestion to provide a chimeric fusion protein to be used in the detection of proANP or proBNP in a sample by, for example, means of an immune assay.

The '838 patent discloses purified BNP fragments and a method for assaying BNP and fragments thereof. This patent discloses precursor molecules of BNP, ANP, CNP, and fragments thereof, but does not include any teaching or suggestion that would guide those of skill in the art to use a fusion polypeptide, either for pharmaceutical or diagnostic purposes.

The '286 patent relates to atrial peptides and analogs thereof, which are used as diuretics, natriuretics, and/or vasodilators, or as intermediates for, or modulators of, such compounds, and methods for the production and use of such compounds. An example of such a compound is atrial natriuretic/vasodilator peptide (ANVP). The '286 patent teaches that ANVP compounds can be synthesized using manual techniques or an automatic peptide synthesizer. Alternatively, the compounds can be produced by expression of recombinant DNA constructs (column 13, lines 30-41). The patent teaches the production of peptides and fragments thereof, but does not teach or suggest production of recombinant fusion polypeptides, as in the present invention. The modification concerns only the amino acid sequences of various forms of pre-proANVP, pro ANVP, and ANVP compounds (column 13, line 55-61). This is shown in the disclosure at column 75, lines 43-55; column 76, lines 56-58; column 77, lines 21-23; column 76, lines 37-41; and column 80, lines 6-10. From these passages, it is clear that the '286 patent does not teach or suggest any fusion polypeptides or peptides including amino acid sequences from different natriuretic peptides, as in the present invention. The compounds produced in the '286 patent are also used to provide immunoassays for the determination of the presence or amount of ANVP compounds in sample (column 86, lines 43-46).

The '576 patent application publication describes a method involving administering to a mammal at risk of, or having, a cardiovascular disease, an amount of a composition including a nucleic acid molecule, e.g., a DNA molecule which encodes BNP, DNP, or chimeras of ANP, CNP, BNP, or DNP (this statement does not disclose whether the chimera comprises different species or a chimera of the same species), effective to inhibit or prevent a cardiovascular disease, e.g., congestive heart failure (page 4, lines 8-12). This is consistent with the teachings of the '619 patent. The peptides of the '576 patent application publication should preferably have an activity similar to or greater than that of BNP, i.e., the peptide is a potent natriuretic, diuretic, vasoactive, and/or lusitropic hormone (page 4, line 33 to page 5, line 2).

The passage on page 4, lines 23-30 further reveals that the chimera is a nucleic acid segment encoding a BNP-chimera or a nucleic acid segment encoding a DNP-chimera. The term "chimeric" is defined to mean that a vector comprises DNA from at least two different species, or comprises DNA from the same species, which is linked or associated in a manner that does not occur in the "native" or wild type of the species (page 22, lines 24-27). More clarity as to what the chimera includes is found on page 5, lines 15-18, which discloses expression cassettes encoding a natriuretic peptide or a chimera thereof, e.g., encoding portions of BNP and DNP.

As with the '619 patent, the '576 patent application publication teaches, at page 5, lines 15-18, that the teachings of the publication provide coding portions of BNP and DNP (see Example 4 and claim 41). This is the only passage that clearly reveals how a humanized, mature form of DNP is prepared from two oligonucleotides synthesized as templates for overlap PCR.

Accordingly, the '576 patent application publication does not propose and would not have encouraged those of skill in the art to make a fusion polypeptide to be used in an

immunoassay for determining the presence of proANP and proBNP in a sample, according to the present invention.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the rejections for obviousness.

Applicants further note that they reserve the right to argue each claim independently, even if this was not done in the present reply.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. Applicants further request an interview with the Examiner prior to the next Action, in the event that the next Action is not a Notice of Allowance. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: April 6, 2010

Susan M. Michaud

Susan M. Michaud, Ph.D.
Reg. No. 42,885

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045